

New record of Shiny Peeping Frog, *Eleutherodactylus nitidus* (Peters, 1870) (Anura, Eleutherodactylidae), in Mexico City, Mexico

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Abstract. Based on molecular and morphological evidence, we report the first record of *Eleutherodactylus nitidus* (Peters, 1870) from Mexico City, in the urban area of Panteón Xilotepec, Xochimilco. We discuss the remarkable urban adaptability of *Eleutherodactylus* and the need for additional sampling to determine if *E. nitidus* is native or introduced to Mexico City.

Key words. Amphibian, distribution, herpetology update, molecular phylogeny, novel occurrence, urban herpetology, *Syrrophus*

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INTRODUCTION

Eleutherodactylus Duméril & Bibron, 1841 is the second most species-rich genus of direct-developing frogs (Anura, Terrarana), with just over 200 recognized species (AmphibiaWeb 2023; Frost 2023). The genus comprises five subgenera (*Eleutherodactylus* Duméril & Bibron, 1841, *Euhyas* Fitzinger, 1843, *Pelorius* Hedges, 1989, *Schwartzius* Hedges, Duellman & Heinicke, 2008, and *Syrrophus* Cope, 1878), of which only *Syrrophus* is not restricted to Caribbean islands (Hedges et al. 2008; Hernández-Austria et al. 2022). In particular, *Syrrophus* includes species with both insular and continental distributions, with two found in western Cuba (*Eleutherodactylus symingtoni* Schwartz, 1957 and *Eleutherodactylus zeus* Schwartz, 1958) (Hernández-Austria et al. 2022; Devitt et al. 2023) and 42 that are cumulatively distributed from the southern United States to Belize and Guatemala, and spanning elevation gradients from sea level to 2400 m above sea level (Reyes-Velasco et al. 2015; Dugo-Cota et al. 2019; Hernández-Austria et al. 2022; Devitt et al. 2023; Grünwald et al. 2023).

Eleutherodactylus (Syrrophus) nitidus (Peters, 1870) is endemic to Mexico, where it has been recorded in the states of Guerrero, Oaxaca, Mexico, Morelos, Tlaxcala, and Puebla (Grünwald et al. 2021; Devitt et al. 2023). While this species has not been previously reported in Mexico City, Lemos-Espinal and Smith (2020) suggested the possibility of its occurrence there due to its distribution in the nearby states of Mexico and Morelos, in particular in the southeast, where a large conservation area covers approximately 59% of the city (Reygadas-Prado 2016).

Here, we use genetic and morphological evidence to verify and confirm an *E. nitidus* specimen from Mexico City, establishing the first published occurrence of this species in this highly urbanized region.



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METHODS

In July 2023 we heard *Eleutherodactylus* calls in the cemetery of Panteón Xilotepec, which is located in Xochimilco, Mexico City. We later collected a specimen on the wall in the cemetery under permit issued to Uri Omar García-Vázquez by the Mexican government's Secretaría de Medio Ambiente y Recursos Naturales (Faut-0246). We euthanized the specimen using 20% benzocaine gel, preserved a liver sample in absolute molecular-grade alcohol, and fixed the specimen in 10% formalin (Beaupre et al. 2004). This specimen is deposited in the Herpetological Collection of the Museo de Zoología, Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México (MZFZ). To verify its taxonomic identity, we compared this specimen with the original descriptions of *E. nitidus* (Peters, 1870), *E. grandis* (Dixon, 1957), and *E.*

maurus (Hedges, 1989). These are the three species of *Eleutherodactylus*, subgenus *Syrrhophus*, with the closest distribution to Mexico City. Our morphological examination focused on the measurements used by Peters (1870): snout to vent length (SVL), head width (HW), diameter of tympanum (TD), diameter of eye (EL), tibia length (TL), and hand length (HAL), averaged with an electronic vernier callipers. In addition, distinctive phenotypic characteristics of the species mentioned by Devitt et al. (2023) and Grünwald et al. (2023) were identified such as: dorsal skin texture, form of canthus rostralis, form of snout, and color pattern.

Additionally, we extracted DNA from the liver sample using a DNeasy Blood & Tissue DNA extraction kit (Qiagen Inc., Valencia, CA, USA). We verified DNA quality by electrophoresis using a 1kb Plus molecular weight marker (100–12,000 bp) and visualized it in an ultraviolet light chamber (UV-Transilluminator). From the extracted DNA, we amplified a fragment of the 16S ribosomal gene through polymerase chain reaction with primers 16Sar (Palumbi 1996) and 16H47 (Heinicke et al. 2007). We purified PCR products using polyethylene glycol (Lis 1980). Finally, the purified sample was sequenced by Macrogen Inc. in South Korea.

To determine its phylogenetic position, we obtained from GenBank 83 sequences of the 16S rRNA gene from the subgenus *Syrrhophus*, including species related to *E. nitidus*, such as *E. grandis*, and *E. maurus*; additionally, we used a sequence of *Eleutherodactylus zeus* Schwartz, 1958 as an outgroup (Appendix, Table A1) (Devitt et al. 2023; Grünwald et al. 2023). We edited the sequence of our new specimen in Sequencher v. 4.1.4 (Gene Code Corporation, USA), and along with the GenBank sequences we aligned it in UGENE 47 (Okonechnikov et al. 2023) using the MUSCLE algorithm (Robert 2004). Subsequently, we used PartitionFinder v. 2 (Lanfear et al. 2017) to choose the partitioning scheme under the greedy search algorithm using a Bayesian Information Criterion. Finally, we constructed a phylogenetic tree in RAxML v. 8.2.X software (Stamatakis 2014) with the graphical interface raxmlGUI v. 2.0 (Edler 2021) using 1000 ultrafast bootstrap replicates to assess branch support.

RESULTS

Eleutherodactylus nitidus (Peters, 1870)

Figures 1–3

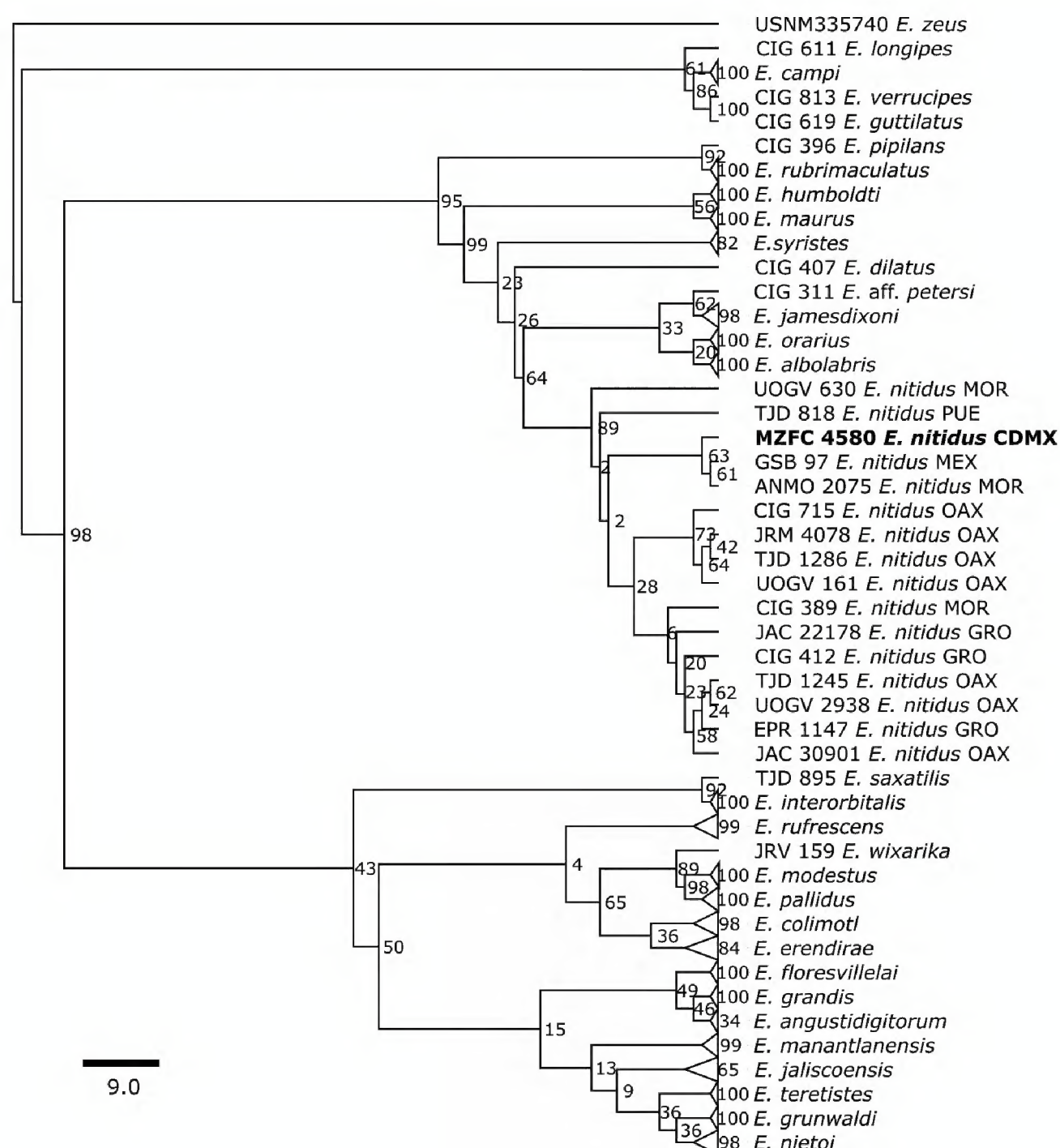
New record. MEXICO – MEXICO CITY • Xochimilco, Panteón Xilotepec; 19.2601°N, 099.1216°W; 2,261 m alt.; 4.VII.2023; D.I. Sánchez-Aguilar, A.G. Montero-Vachier, V.G. Castillo-Sánchez, U.O. García-Vázquez leg.; hidden inside a crevice of a wall; 1 ♂, MZfZ 4580.

Identification. The morphological characters of the specimen agree with the original description of *E. nitidus* by Peters (1870). Important morphological measurements of the specimen are as follows: SVL = 22.09 mm, HW = 6.56 mm, TD = 0.66 mm, EL = 2.70 mm, TL = 8.63 mm, and HAL = 6.89 mm. Additional diagnostic characteristics include: (1) granular dorsum, (2) rounded canthus rostralis, (3) rounded tip of snout, (4)

Figure 1. *Eleutherodactylus nitidus* (Peters, 1870) specimen collected from Panteón Xilotepec, Xochimilco, Mexico City (MZfZ 4580). **A.** Full body view. **B.** Front view. **C.** Lateral view. **D.** Ventral view.



Figure 2. Phylogenetic relationships of the 16S gene obtained from the maximum-likelihood analysis of the members of *Eleutherodactylus* subgenus *Syrrhophus*. Numbers in the nodes represent the bootstrap support value. All nodes at species level were collapsed except for *E. nitidus*.



tympanum barely visible through the skin, (5) mottled lip margins, and (6) brown-banded limbs (Figure 1). In life, the dorsal coloration is green, with a brownish tone due to skin granules. The green coloration is more intense on the extremities, which also have brown bands. The lumbar gland is white with black spots that extend laterally on both sides of the body. The venter is pale, as are the legs (Figure 1). In comparison to *E. grandis*, our new specimen is smaller in size (*E. grandis*: SVL = 30.5 mm), with a narrower head (*E. grandis*: HW = 11.7 mm), and a smaller tympanum (*E. grandis*: TD = 2 mm). The latter characteristic of the specimen collected is shared with *E. maurus* (a small, inconspicuous tympanum), but unlike the latter it lacks a black line anterior and posterior to the eye (present in *E. maurus*), has a mostly white lumbar gland (mostly black in *E. maurus*), and has pale lips with greenish spots (dark lips with pale speckles in *E. maurus*) (Peters 1870; Davis and Dixon 1955; Dixon 1957; Devitt et al. 2023; Grünwald et al. 2023).

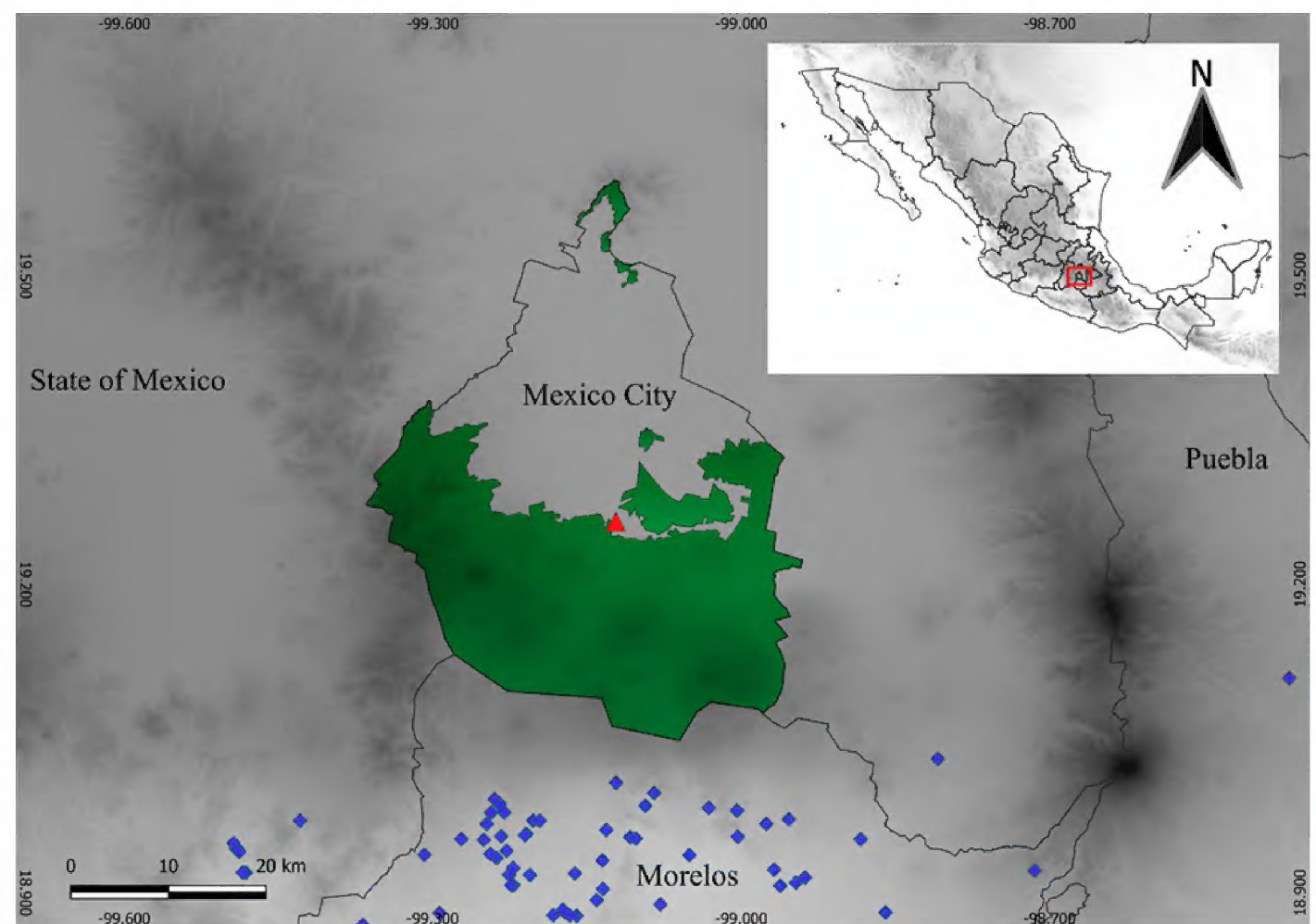
Molecular analysis. We included 84 sequences in our phylogenetic analysis, and in the final alignment we obtained a matrix of 598 base pairs for the 16S rRNA gene across 32 species of the genus.

We recovered a tree topology similar to prior phylogenies of *Syrrhophus* (Grünwald et al. 2018; Devitt et al. 2023; Figure 2), with three major groups. The first major group (bootstrap support [bs] = 61) is made up of *E. verrucipes* as the sister taxon to *E. guttilatus* (bs = 100), which in turn are sister group to *E. campi* (bs = 86), and which cumulatively form the sister group to *E. longipes* (bs = 61). The second major group consists of the genetic samples of *E. nitidus* (bs = 89), forming a monophyletic group that is sister to a cluster of other species within the genus (bs = 64). These species include *E. aff. petersi*, *E. jamesdixoni*, *E. orarius*, and *E. albolabris*, which collectively serve as the sister group to *E. dilatatus* (bs = 26). *Eleutherodactylus dilatatus*, in turn, is sister to *E. syristes* (bs = 23), which is further sister to genetic samples of *E. humboldti* and *E. maurus* (bs = 99). Finally, this clade is sister to the genetic samples of *E. pipilans* and *E. rubrimaculatus* (bs = 95). The third major clade (bs = 98) comprises *E. saxatilis*, *E. interorbitalis*, *E. rufescens*, *E. wixarika*, *E. modestus*, *E. pallidus*, *E. colimotl*, *E. erendirae*, *E. floresvillelai*, *E. grandis*, *E. angustidigitorum*, *E. manantlanensis*, *E. jaliscoensis*, *E. teretistes*, *E. grunwaldi*, and *E. nietoi*.

The new specimen collected in Mexico City (MZFC 4580) grouped within the *E. nitidus* clade and is sister to the genetic samples from the geographically proximate State of Mexico and Morelos (bs = 63) (Figure 2).

Additional records. At the site where we collected the specimen, other *Eleutherodactylus* calls were heard, indicating the presence of more individuals (Supplemental data, Audio File S1).

Figure 3. *Eleutherodactylus nitidus* in Mexico City, and closest records. MZFZ 4580 (red triangle); literature records (blue diamonds). The green area represents the conservation area of Mexico City.



DISCUSSION

The origin of *Eleutherodactylus nitidus* in Mexico City is uncertain. The examined records might be native due to their proximity and phylogenetic affinity to records from the State of Mexico and Morelos. The distance between our new record and closest previously known occurrence in Morelos, which borders the southern part of Mexico City, is 27 km. Here, in this part of the city, Lemos-Espinal and Smith (2020) speculated that *E. nitidus* might be present in forests and ravines. However, human transport cannot be ruled out, because Kraus et al. (1999) and Kaiser et al. (2002) mentioned that *Eleutherodactylus* species have been introduced through the trade of ornamental plants.

Although most specimens are collected from wildland areas, urban parks are increasingly recognized as conducive to maintaining *Syrrhophus* populations. The documented adaptability of *E. nitidus* to urban settings has been underscored in prior studies. This adaptability has been attributed to these frogs' small size and to their direct developing life history, which frees them from requiring large areas of habitat or standing water to survive and reproduce (García-Vázquez and Trujano-Ortega 2012; Gómez-Benitez et al. 2021; Vázquez-Hernández 2023). Our new record from Mexico City adds to this steadily growing documentation of *E. nitidus* from highly urbanized areas.

In the absence of definitive historical data to classify species as native or non-native to an area, phylogeographic and genetic analyses are valuable tools. These analyses can reveal genetic signatures of the founder effect for introduced populations, which often have reduced genetic variation due to rapid population expansion, have more recent divergence dates, and are less likely to be genetically structured compared to populations from their native range (Yuan et al. 2022). Records of the genus in the city may be misidentified as *E. grandis*, given the restricted distribution of the species (Serrano-Serrano, 2016). To know how many species of the genus are currently in the city and to decide if *E. nitidus* is native or non-native, an extensive sampling is necessary across a larger geographic area.

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Authors contributions


Conceptualization: UOGV. Formal analysis: DISA, VGCS. Funding acquisition: UOGV Investigation: DISA, VGCS, AGMV, UOGV. Methodology: DISA, VGCS, AGMV. Resources: DISA, VGCS, AGMV. Supervision: UOGV. Visualization: DISA, UOGV. Project administration: DISA, UOGV. Validation: UOGV. Writing – original draft: DISA, VGCS, AGMV. Writing – review and editing: DISA, VGCS, AGMV, UOGV.

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Data availability

All data that support the findings of this study are available in the main text and Supplementary data.

Supplementary data

Audio File S1. Calls of *Eleutherodactylus* heard at Panteón Xilotepec, Xochimilco, Mexico City (mp4 format).

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APPENDIX

Table A1. List of all the terminal taxa and sequences by gene used in the present study, accession numbers of sequences retrieved from GenBank, and new sequences produced for this study.

Taxa	Field no.	Collection no.	GenBank accession	Latitude	Longitude	State	Reference
<i>E. nitidus</i>	UOGV 4994	MZFC 4580	PP832450	19.2610	−099.1213	Mexico City	This study
<i>E. albolabris</i>	CIG 391	MZFC 33083	MG856955	17.3289	−099.4698	Guerrero	Grünwald et al. 2018
	CIG 953	MZFC 33323	MG856956	17.3289	−099.4698	Guerrero	Grünwald et al. 2018
<i>E. angustidigitum</i>	CIG 378	MZFC 33070	MG856962	19.6678	−100.9052	Michoacán	Grünwald et al. 2018
	CIG 479	MZFC 33130	MG856964	19.9801	−103.7868	Jalisco	Grünwald et al. 2018
<i>E. campi</i>	CIG 606	MZFC 33195	MG856965	25.3264	−100.2201	Nuevo León	Grünwald et al. 2018
	CIG 609	MZFC 33198	MG856966	25.3264	−100.2201	Nuevo León	Grünwald et al. 2018
<i>E. colimotl</i>	CIG 340	MZFC 33036	MG856967	18.5822	−103.5222	Michoacán	Grünwald et al. 2018
	CIG 462	MZFC 33115	MG856971	19.0617	−103.7811	Colima	Grünwald et al. 2018

Taxa	Field no.	Collection no.	GenBank accession	Latitude	Longitude	State	Reference
<i>E. colimotl</i>	JAC 23999	UTA A-64020	OQ145187	18.5850	−103.5162	Michoacán	Devitt et al. 2023
	JAC 24001	UTA A-64022	OQ145189	18.5850	−103.5162	Michoacán	Devitt et al. 2023
<i>E. dilatus</i>	CIG 407	MZFC 33091	MG856973	17.5566	−099.6721	Guerrero	Grünwald et al. 2018
<i>E. erendirae</i>	CIG 303	MZFC 33003	MG856975	19.6853	−102.9245	Jalisco	Grünwald et al. 2018
	CIG 306	MZFC 33006	MG856976	19.6853	−102.9245	Jalisco	Grünwald et al. 2018
	CIG 308	MZFC 33008	MG856977	19.6853	−102.9245	Jalisco	Grünwald et al. 2018
	CIG 319	MZFC 29274	MG856980	19.3379	−102.2698	Michoacán	Grünwald et al. 2018
	CIG 320	MZFC 33019	MG856981	19.3379	−102.2698	Michoacán	Grünwald et al. 2018
<i>E. floresvillelai</i>	CIG 361	MZFC 33053	MG856983	19.6502	−100.9452	Michoacán	Grünwald et al. 2018
	CIG 366	MZFC 33058	MG856986	19.6502	−100.9452	Michoacán	Grünwald et al. 2018
<i>E. grandis</i>	TJD 831	IBH 34814	MG856989	19.3177	−099.1971	Mexico City	Grünwald et al. 2018
	TJD 832	IBH 34815	OQ145196	19.3177	−099.1971	Mexico City	Devitt et al. 2023
<i>E. grunwaldi</i>	JRV 139	MZFC 27472	MG856990	19.4316	−103.9787	Colima	Grünwald et al. 2018
	JRV 255	Uncatalogued	MG856991	—	—	Jalisco	Grünwald et al. 2018
<i>E. guttilatus</i>	CIG 619	MZFC 33200	MG856994	23.5838	−100.8909	San Luis Potosí	Grünwald et al. 2018
<i>E. humboldti</i>	TJD 1307	MZFZ 4505	OQ145202	19.0943	−100.0827	México	Devitt et al. 2023
	TJD 1308	MZFZ 4506	OQ145203	19.1538	−100.14292	México	Devitt et al. 2023
<i>E. interorbitalis</i>	CIG 584	MZFC 33186	MG856995	23.7358	−106.5962	Sinaloa	Grünwald et al. 2018
	CIG 585	MZFC 33187	MG856996	23.7358	−106.5962	Sinaloa	Grünwald et al. 2018
<i>E. jaliscoensis</i>	CIG 480	MZFC 33131	MG856998	20.2191	−104.7357	Jalisco	Grünwald et al. 2018
	CIG 482	MZFC 33133	MG857005	20.2191	−104.7357	Jalisco	Grünwald et al. 2018
	CIG 486	MZFC 33137	MG857000	20.2138	−104.7307	Jalisco	Grünwald et al. 2018
	CIG 490	MZFC 33141	MG856997	20.2085	−104.7478	Jalisco	Grünwald et al. 2018
	CIG 861	MZFC 33274	MG857002	20.6980	−104.8671	Jalisco	Grünwald et al. 2018
<i>E. jamesdixonii</i>	CIG 687	MZFC 33242	MG857035	21.2800	−104.6800	Nayarit	Grünwald et al. 2018
	TJD 896	IBH 34851	OQ145212	23.5042	−105.8370	Sinaloa	Devitt et al. 2023
	TJD 897	IBH 34852	OQ145213	23.5041	−105.8370	Sinaloa	Devitt et al. 2023
<i>E. longipes</i>	CIG 611	MZFC 33199	MG857006	25.3072	−100.2175	Nuevo León	Grünwald et al. 2018
<i>E. manantlanensis</i>	CIG 893	MZFC 33293	MG857007	19.4414	−103.9583	Colima	Grünwald et al. 2018
	CIG 895	MZFC 33295	MG857008	19.4414	−103.9583	Colima	Grünwald et al. 2018
	JRV 144	MZFC 27476	MG857009	19.4346	−103.9595	Colima	Grünwald et al. 2018
<i>E. maurus</i>	CIG 380	MZFC 33072	MG857011	18.9830	−099.3221	México	Grünwald et al. 2018
	CIG 388	MZFC 33080	MG857010	19.0160	−099.2652	Morelos	Grünwald et al. 2018
<i>E. modestus</i>	CIG 891	MZFC 33291	MG857012	19.2699	−104.0749	Colima	Grünwald et al. 2018
	JAC 23846	Uncatalogued	MG857013	-	—	Jalisco	Grünwald et al. 2018
<i>E. nietoi</i>	CIG 346	MZFC 33042	MG857022	18.6989	−103.4115	Michoacán	Grünwald et al. 2018
	CIG 348	MZFC 33044	MG857023	18.6989	−103.4115	Michoacán	Grünwald et al. 2018
	CIG 974	MZFC 33336	MG857026	18.6666	−099.5191	Michoacán	Grünwald et al. 2018
	CIG 975	MZFC 33337	MG857027	18.6666	−103.4281	Michoacán	Grünwald et al. 2018
<i>E. nitidus</i>	ANMO 2075	MZFC 23404	OQ145223	18.6763	−099.5345	Morelos	Devitt et al. 2023
	CIG 389	MZFC 33081	MG857029	18.5648	−099.1884	Morelos	Grünwald et al. 2018
	CIG 412	MZFC 33096	MG857031	17.5547	−099.6243	Guerrero	Grünwald et al. 2018
	CIG 715	MZFC 33248	MG857030	17.0200	−097.7700	Oaxaca	Grünwald et al. 2018
	EPR 1147	MZFC 11548	OQ145225	17.6997	−099.5191	Guerrero	Devitt et al. 2023
	GSB 97	Uncatalogued	OQ145226	18.8460	−099.9864	México	Devitt et al. 2023
	JAC 22178	UTA A-56515	OQ145228	17.6595	−099.7021	Guerrero	Devitt et al. 2023
	JAC 30901	Uncatalogued	OQ145231	17.6272	−097.3310	Oaxaca	Devitt et al. 2023
	JRM 4078	MZFC 10611	OQ145234	16.6684	−094.1925	Oaxaca	Devitt et al. 2023
	TJD 1245	MZFZ 4486	OQ145246	17.5182	−097.5156	Oaxaca	Devitt et al. 2023
	TJD 1286	MZFZ 4501	OQ145254	16.9226	−096.3468	Oaxaca	Devitt et al. 2023
	TJD 818	IBH 34809	OQ145237	18.6042	−098.4867	Puebla	Devitt et al. 2023
	UOGV 161	Uncatalogued	OQ145255	17.8876	−096.9012	Oaxaca	Devitt et al. 2023
	UOGV 2938	MZFZ 4507	OQ145257	17.6527	−097.9539	Oaxaca	Devitt et al. 2023
	UOGV 630	MZFC 23342	OQ145256	18.6000	−099.1000	Morelos	Devitt et al. 2023

Taxa	Field no.	Collection no.	GenBank accession	Latitude	Longitude	State	Reference
<i>E. orarius</i>	CIG 341	MZFC 33037	MG857041	18.5822	−103.5222	Michoacán	Grünwald et al. 2018
	CIG 460	MZFC 33113	MG857042	19.0264	−103.7903	Colima	Grünwald et al. 2018
<i>E. pallidus</i>	CIG 586	MZFC 33188	MG857043	23.4004	−105.9156	Sinaloa	Grünwald et al. 2018
	CIG 650	MZFC 33212	MG857048	21.4600	−105.0300	Nayarit	Grünwald et al. 2018
	CIG 858	MZFC 33271	MG857044	20.6371	−104.8601	Jalisco	Grünwald et al. 2018
<i>E. pipilans</i>	CIG 396	MZFC 33086	MG857054	17.3800	−099.4789	Guerrero	Grünwald et al. 2018
<i>E. rubrimaculatus</i>	CIG 753	MZFC 33249	MG857056	15.0442	−092.0897	Chiapas	Grünwald et al. 2018
	CIG 755	MZFC 33250	MG857057	15.2286	−092.4741	Chiapas	Grünwald et al. 2018
<i>E. rufescens</i>	CIG 345	MZFC 33041	MG857068	18.7327	−103.2936	Michoacán	Grünwald et al. 2018
	CIG 551	MZFC 33172	MG857063	19.4746	−103.2577	Jalisco	Grünwald et al. 2018
	JRV 184	MZFC 26908	MG857040	19.5318	−103.5271	Jalisco	Grünwald et al. 2018
	JRV 85	MZFC 26887	MG857036	19.7104	−102.9299	Jalisco	Grünwald et al. 2018
<i>E. saxatilis</i>	TJD 895	IBH 34850	MG857069	23.5042	−105.8368	Sinaloa	Grünwald et al. 2018
<i>E. aff. petersi</i>	CIG 311	MZFC 33011	MG857033	19.6497	−102.9184	Jalisco	Grünwald et al. 2018
<i>E. syristes</i>	CIG 628	MZFC 33208	MG857073	15.9381	−096.6084	Oaxaca	Grünwald et al. 2018
	CIG 954	MZFC 33324	MG857070	17.3289	−099.4698	Guerrero	Grünwald et al. 2018
<i>E. teretistes</i>	CIG 491	MZFC 33142	MG857074	20.2105	−104.7524	Jalisco	Grünwald et al. 2018
	CIG 492	MZFC 33143	MG857075	20.2105	−104.7524	Jalisco	Grünwald et al. 2018
<i>E. verrucipes</i>	CIG 813	MZFC 33253	MG857079	23.5823	−099.7484	Tamaulipas	Grünwald et al. 2018
<i>E. wixarika</i>	JRV 159	MZFC 27477	MG857080	22.0594	−103.9339	Jalisco	Grünwald et al. 2018
<i>E. zeus</i>	172582	USNM 335740	EF493718	22.4111	−083.6890	Cuba	Heinicke et al. 2007